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title: DEVICE AND METHOD FOR  
IN-LINE BLOOD TESTING USING  
BIOCHIPS

Inventor(s): David CHIEN et al.  
DOCKET NO.: 072121-0371

Diagram 1

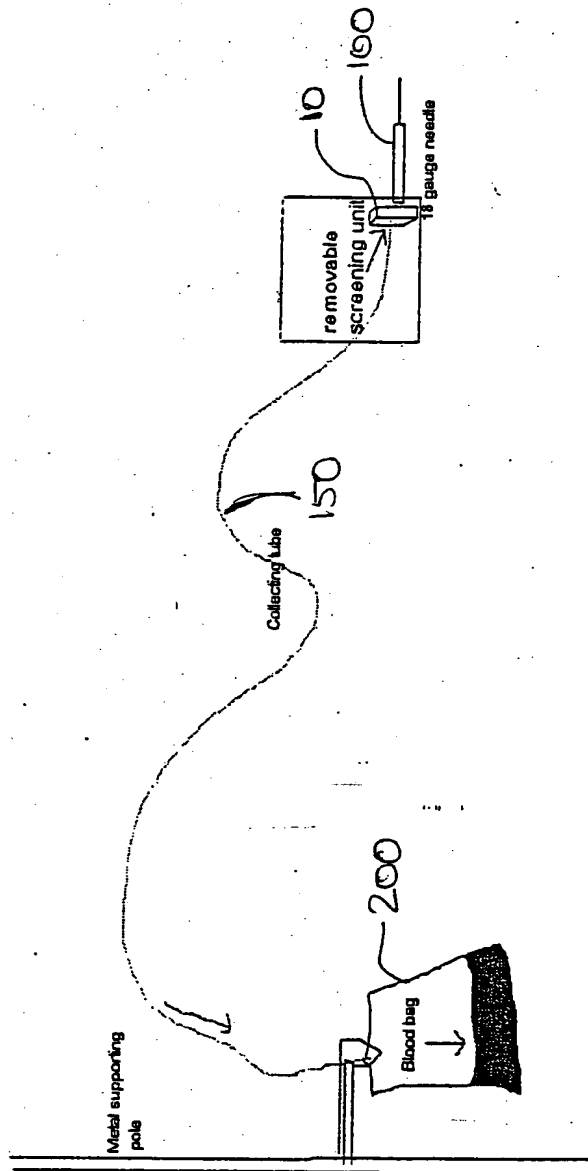
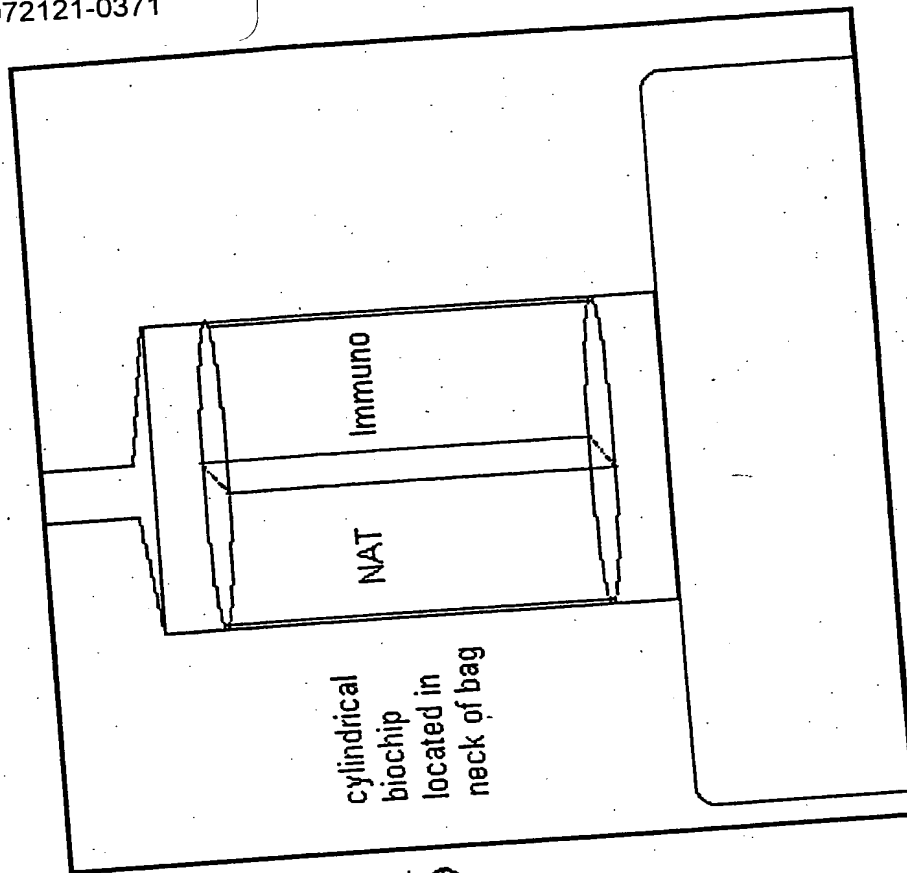


FIGURE 1A



Once collection of initial 10 to 40 ml is complete, the tube leading to that bag will be clamped and sealed off using a thermo sealer, while the clamp leading to the second bag will open. The bag with the biochip will then be put on a slow shaker to maximize contact of biochip with blood.

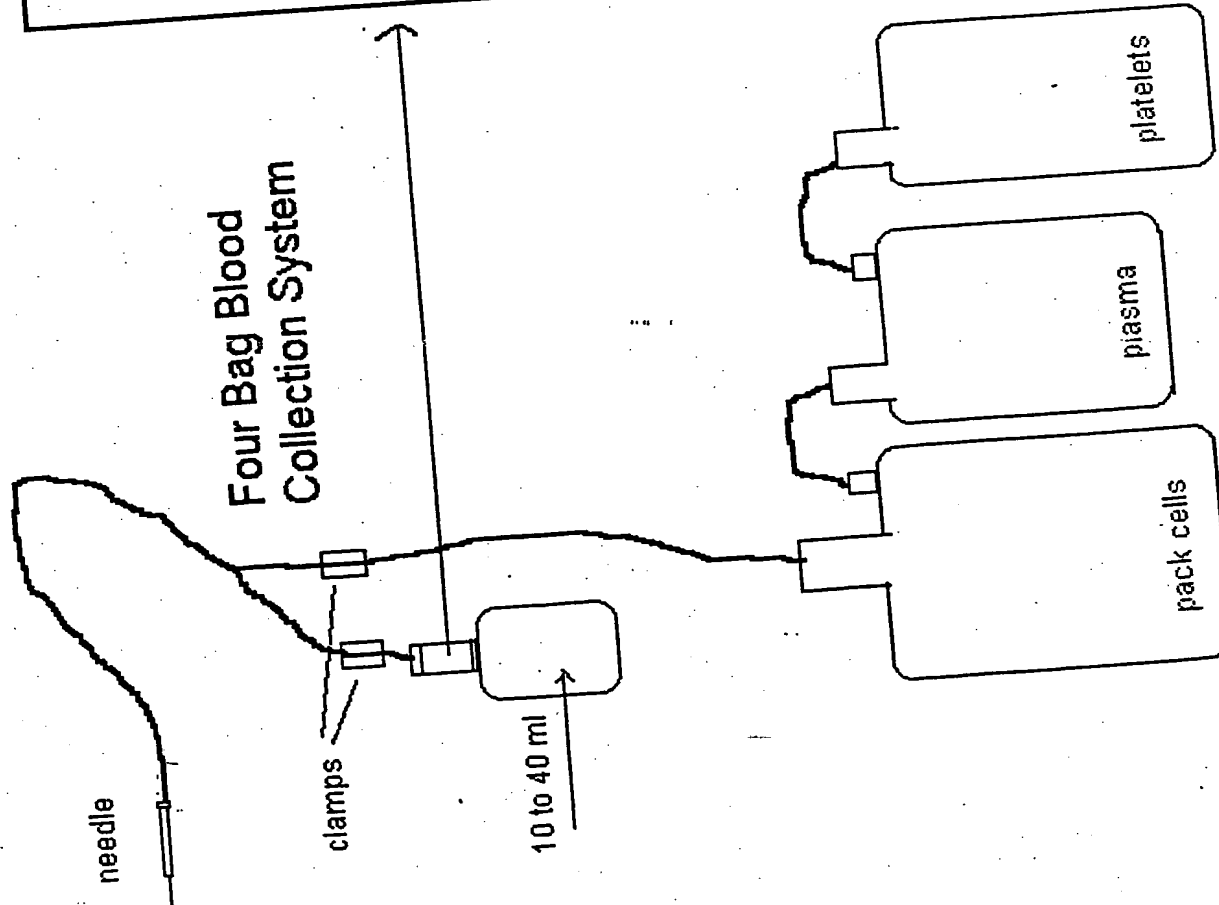


FIGURE 1B

title: DEVICE AND METHOD FOR  
IN-LINE BLOOD TESTING USING  
BIOCHIPS

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Diagram 2

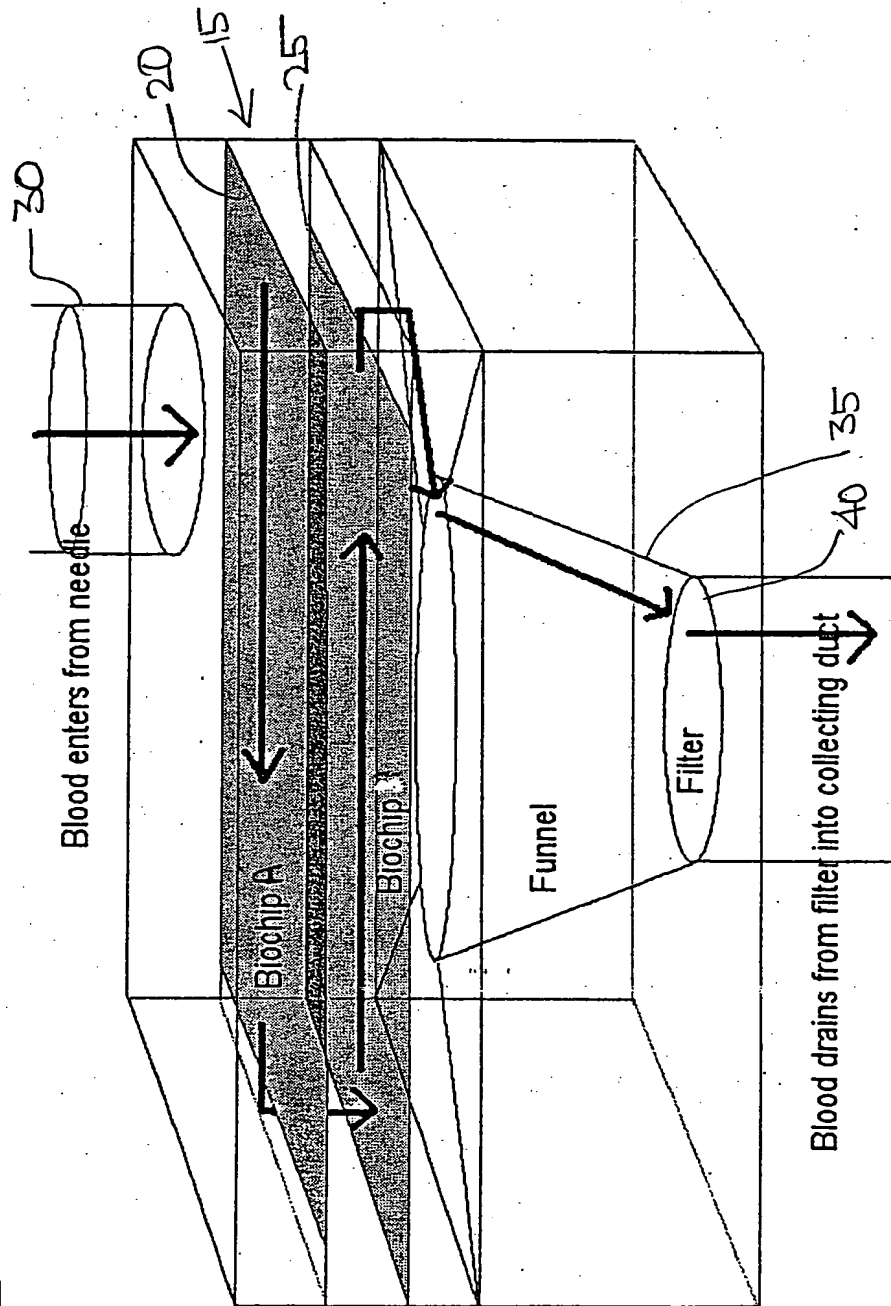


FIGURE 2

title: DEVICE AND METHOD FOR  
IN-LINE BLOOD TESTING USING  
BIOCHIPS

Inventor(s): David CHIEN et al.  
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Diagram 3

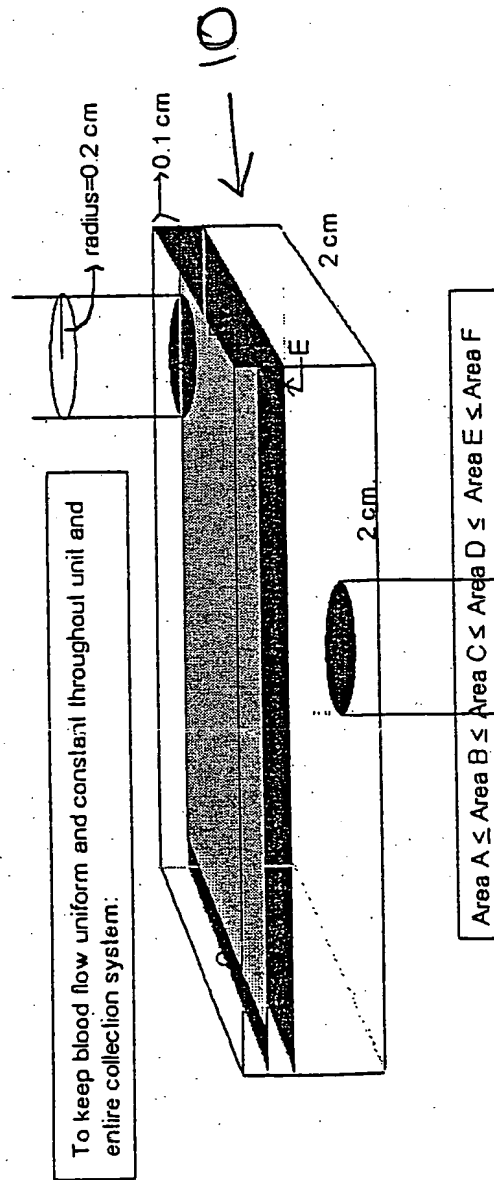


FIGURE 3

title: DEVICE AND METHOD FOR  
IN-LINE BLOOD TESTING USING  
BIOCHIPS

Inventor(s): David CHIEN et al.  
DOCKET NO.: 072121-0371

Diagram 4

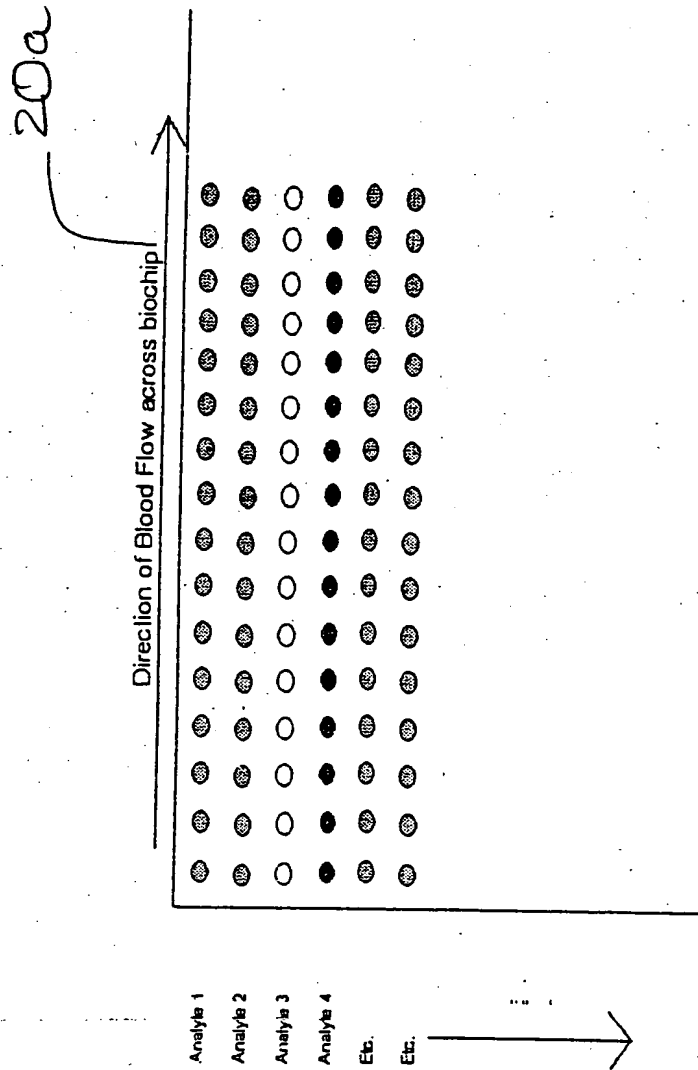
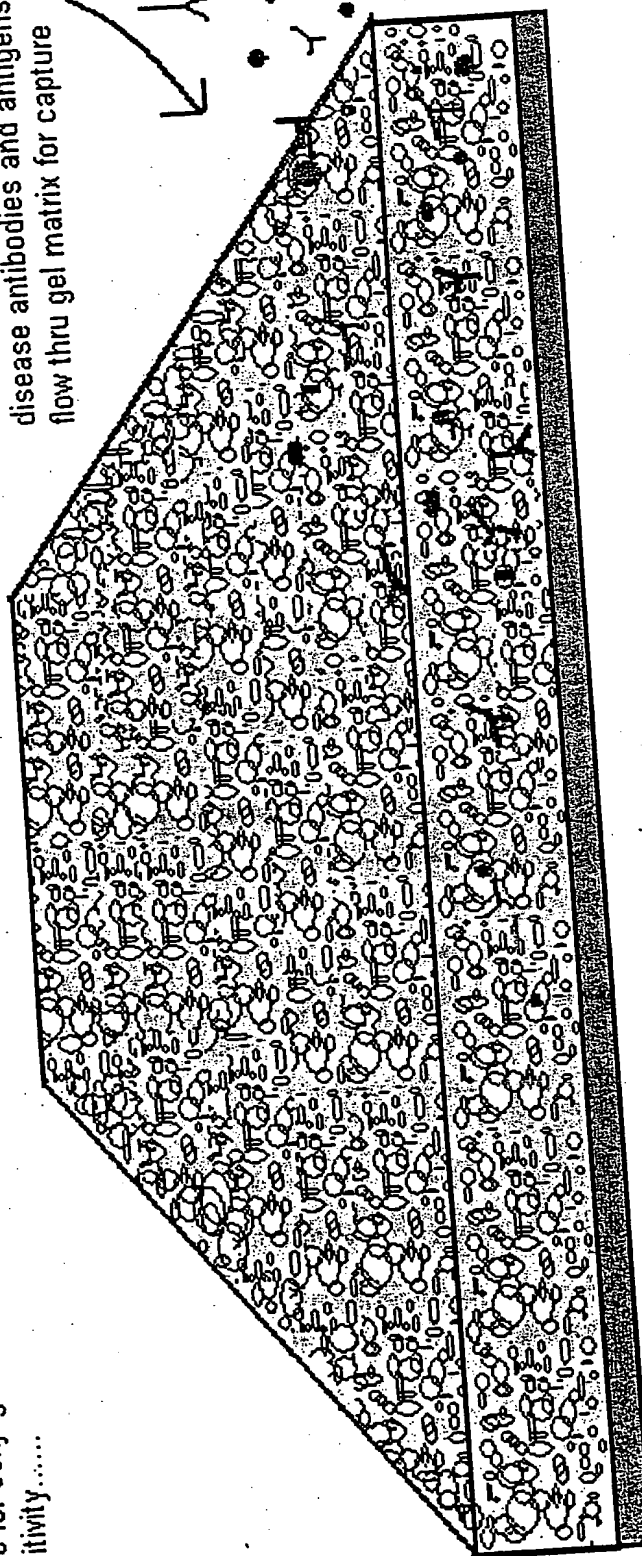


FIGURE 4A

Hypogel technology - 3D matrix to enhance  
space for conjugation/capture, as well as  
sensitivity.....

disease antibodies and antigens  
flow thru gel matrix for capture

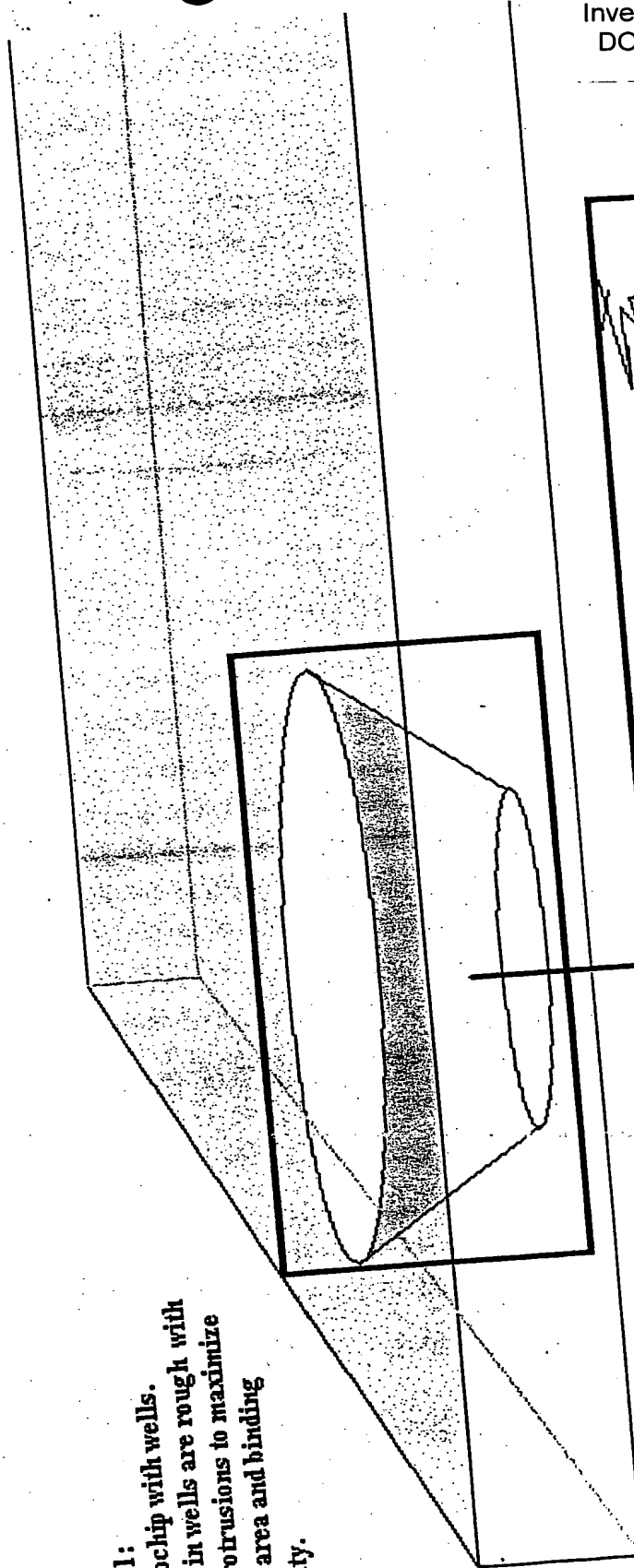


HypoGel® is a hydrophilic polystyrene gel-type resin.

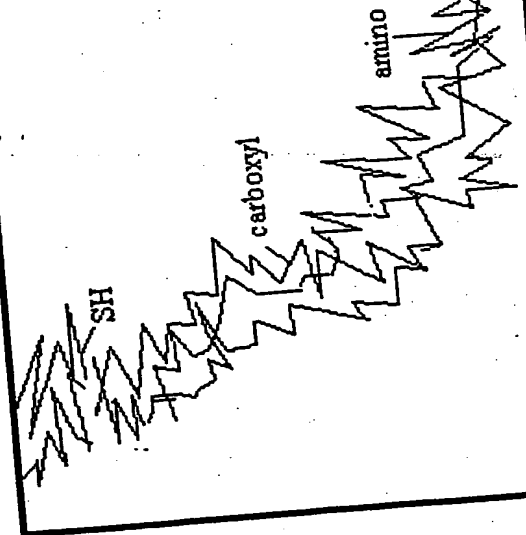
Based on a low crosslinked (1% DVB) polystyrene matrix, oligo ethylene glycols are grafted to form a high loaded hydrophilic resin. The reactive centers are located at the terminus of the glycol spacers. NMR measurements indicate their high flexibility.

FIGURE 4B

**Version 1:**  
Glass biochip with wells.  
Surface in wells are rough with  
small protrusions to maximize  
surface area and binding  
capability.

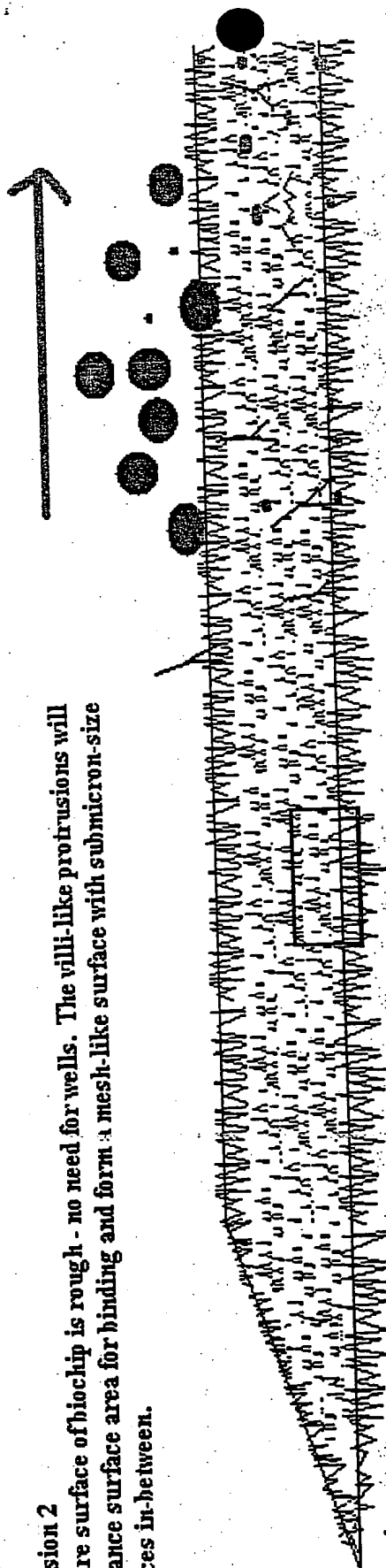


On the glass surface are different  
functional groups attached by  
chemical linking, to allow for different  
binding chemistry to occur with  
disease agents.  
The spaces provided by this mesh of  
polystyrene or glass should be  
submicron in size, since we don't want  
red blood cells caught.



# Version 2

Entire surface of biochip is rough - no need for wells. The villi-like protrusions will enhance surface area for binding and form a mesh-like surface with submicron-size spaces in-between.



Red blood cells will flow pass these inlets, as they are of submicron size. Also, the varying functional groups will allow for different binding chemistry on the biochip.

Different functional groups will be bound by chemical linking to the glass or polystyrene surface.

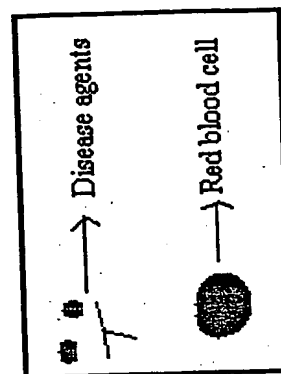
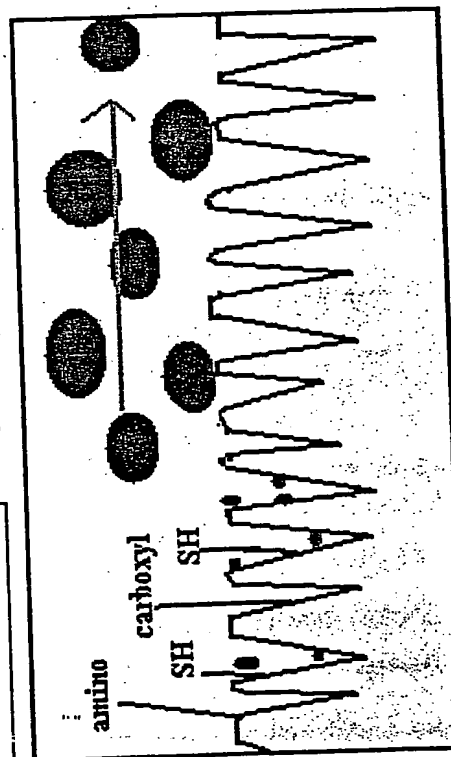
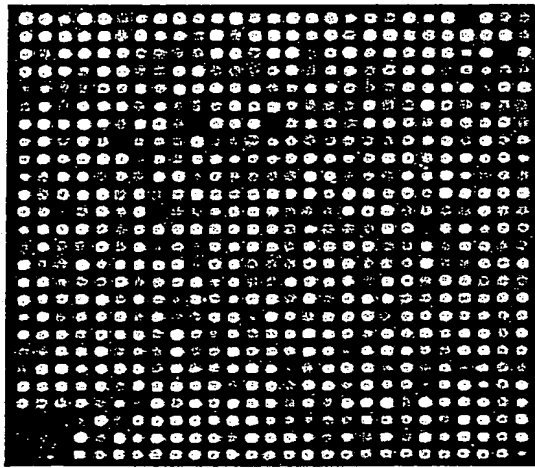


FIG. 4D

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Example of a biochip by Rockefeller University's Gene Array Resource Center ([www.rockefeller.edu](http://www.rockefeller.edu))



← 20

Analytes to be spotted on Biochip #1 (NAT)

HCV: mAb against core (C22)  
mAb against env E1E2  
mAb against NS3, NS4, NS5

HIV: mAb against GP120  
mAb against P24 core  
mAb against P55 core  
mAb against P31

HBV: mAb against core mAb against HBsAg  
mAb against HBeAg mAb against S1 and S2

Also, nucleic acids themselves will be spotted for hybridization:

HCV: 5' end NTR; HIV: LTR region, pol gene region; HBV: preS1/2 and S region

← 22

FIGURE 5

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Diagram 5

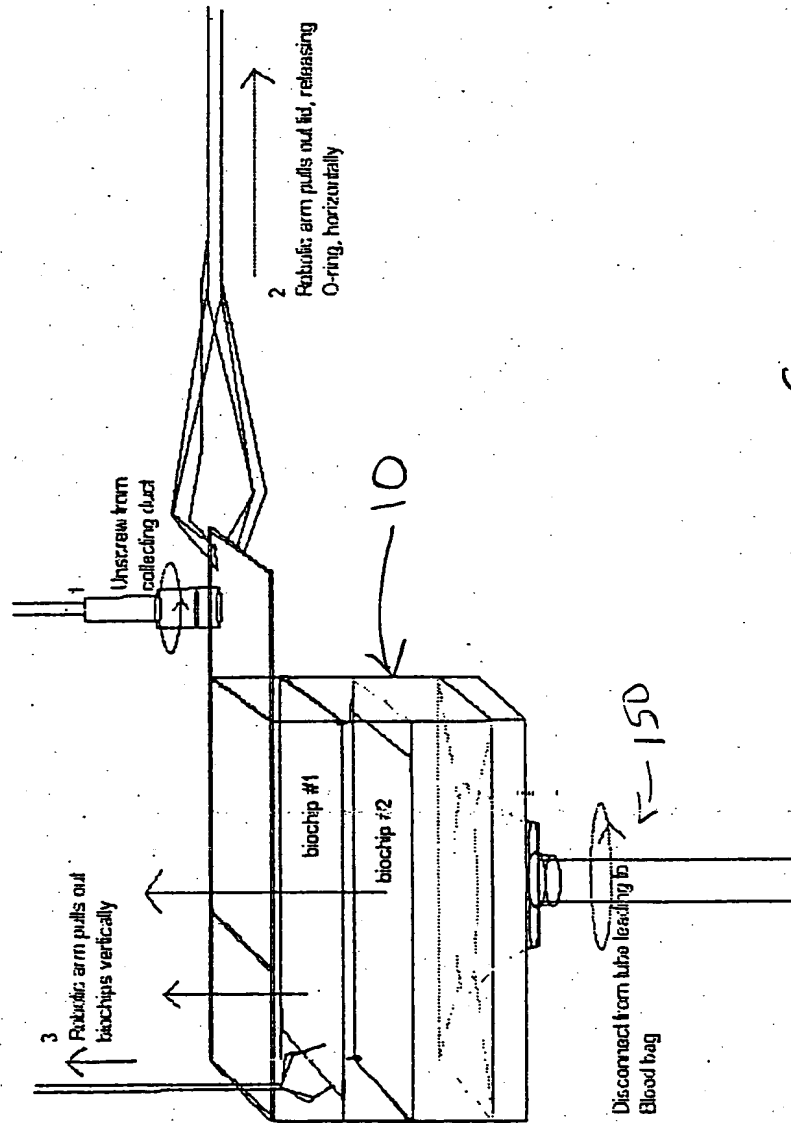


FIGURE 6

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BIOCHIPS

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Diagram 6

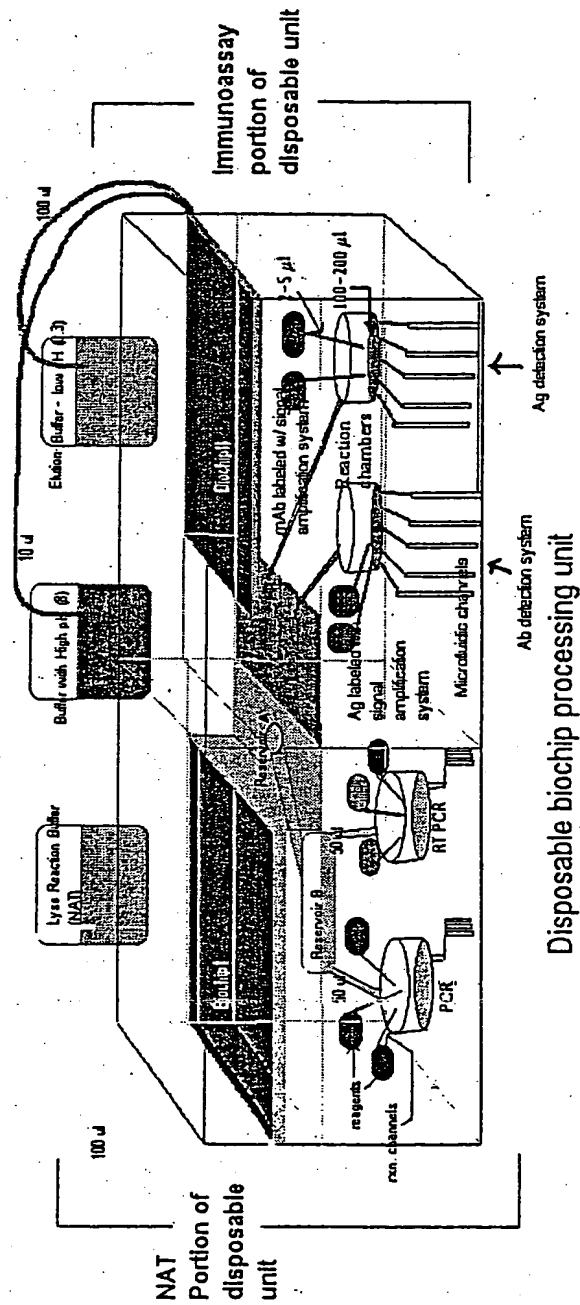


FIGURE 7A

# Alternate design for disposable biochip processor:

Chip I and Chip II are combined in one. Instead of running reagents for each in parallel, they will run in series, leading ultimately to two separate portions: NAT and Immunoassay.

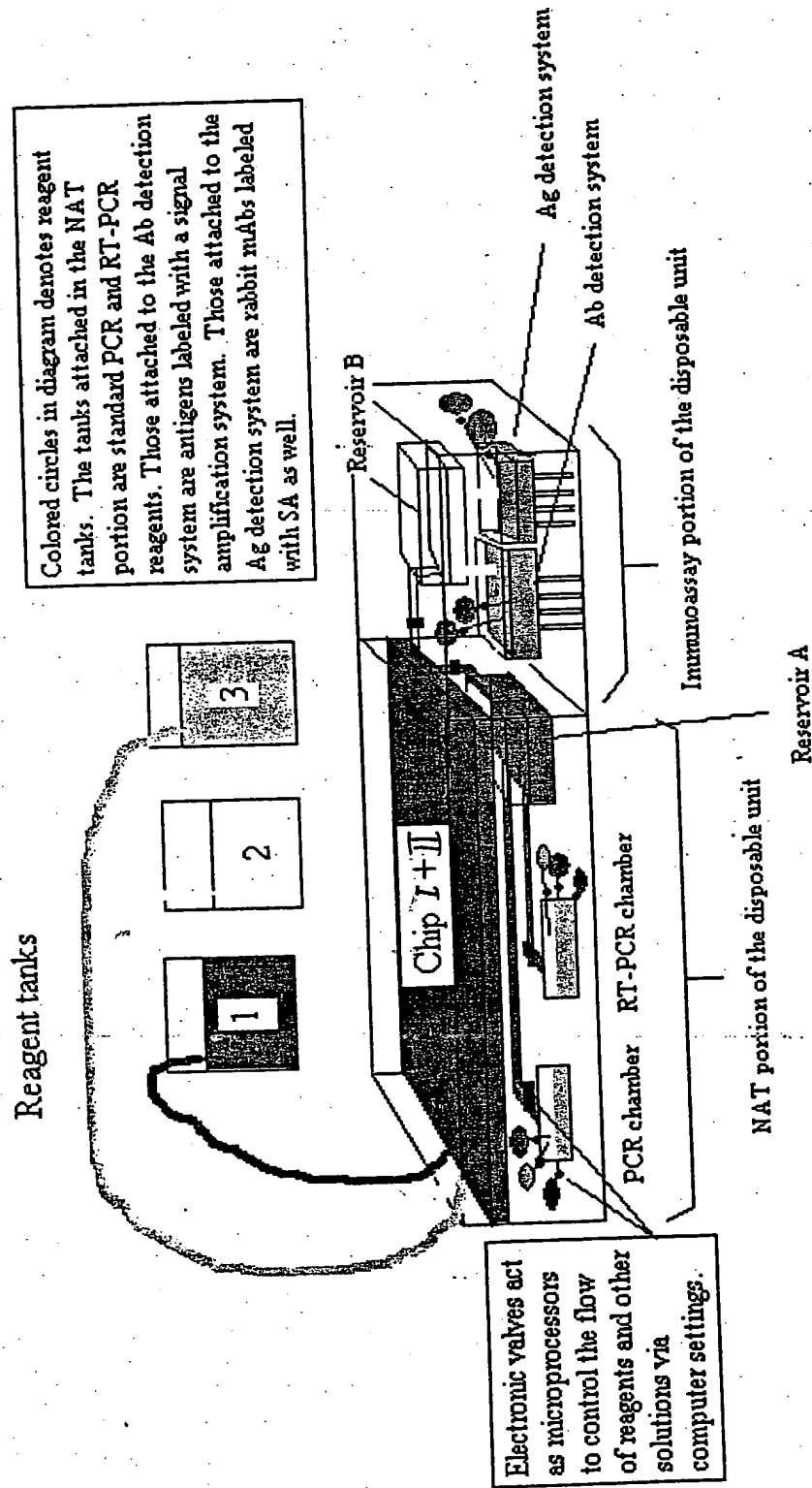
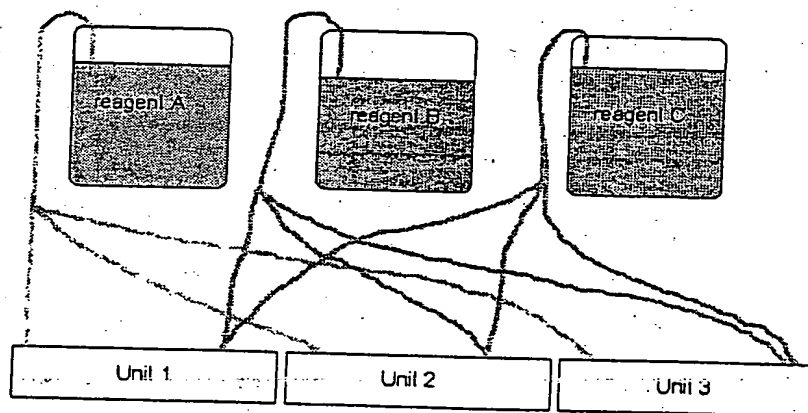


FIGURE 7B

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Diagram 7



In theory, hundreds of these disposable units  
could be hooked up to the reagent dispensers and  
run in parallel.....

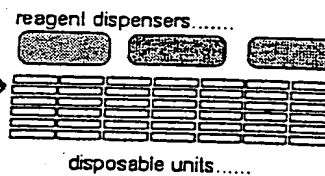


FIGURE 8

Diagram 8

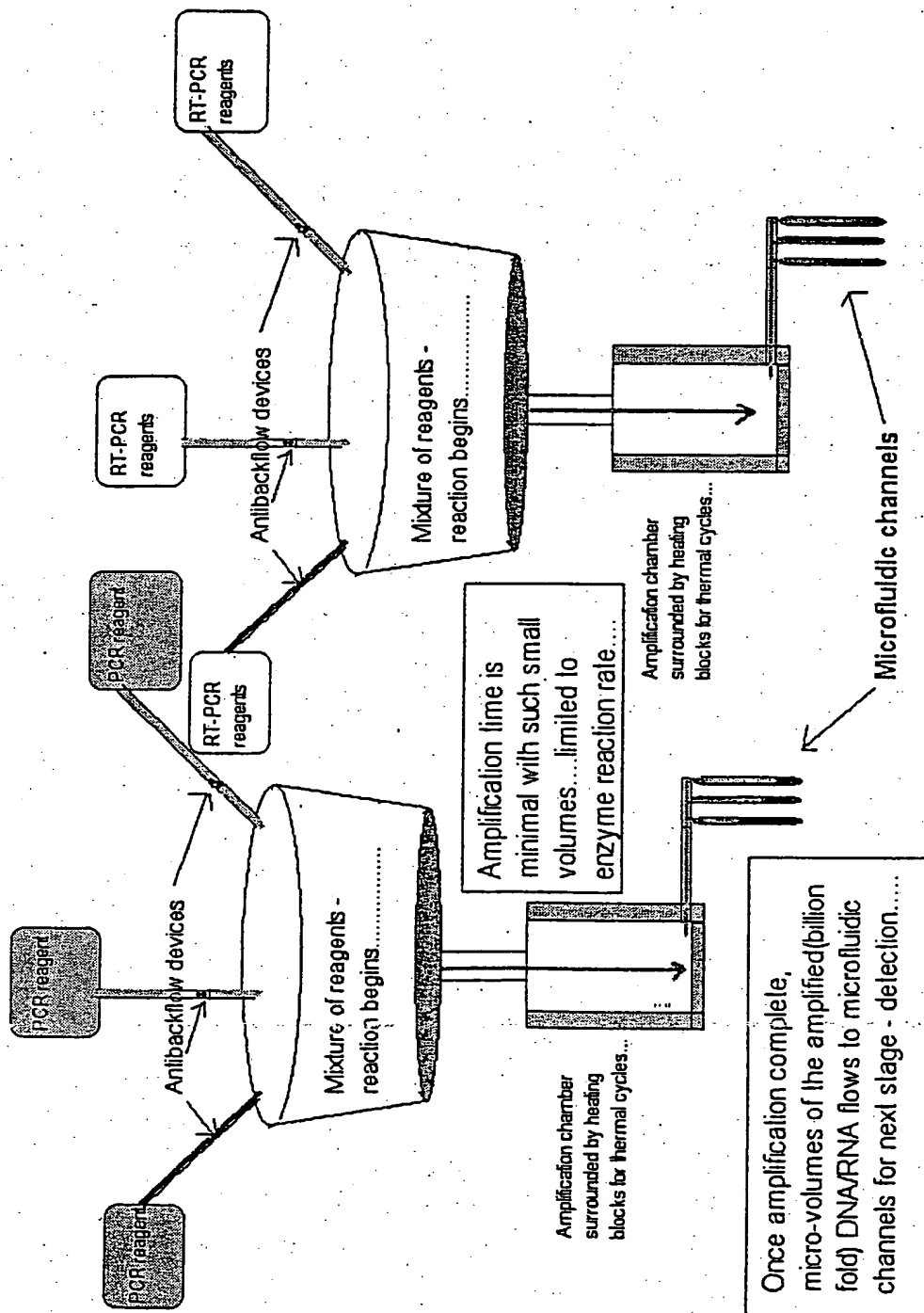


FIGURE 9

Diagram 9

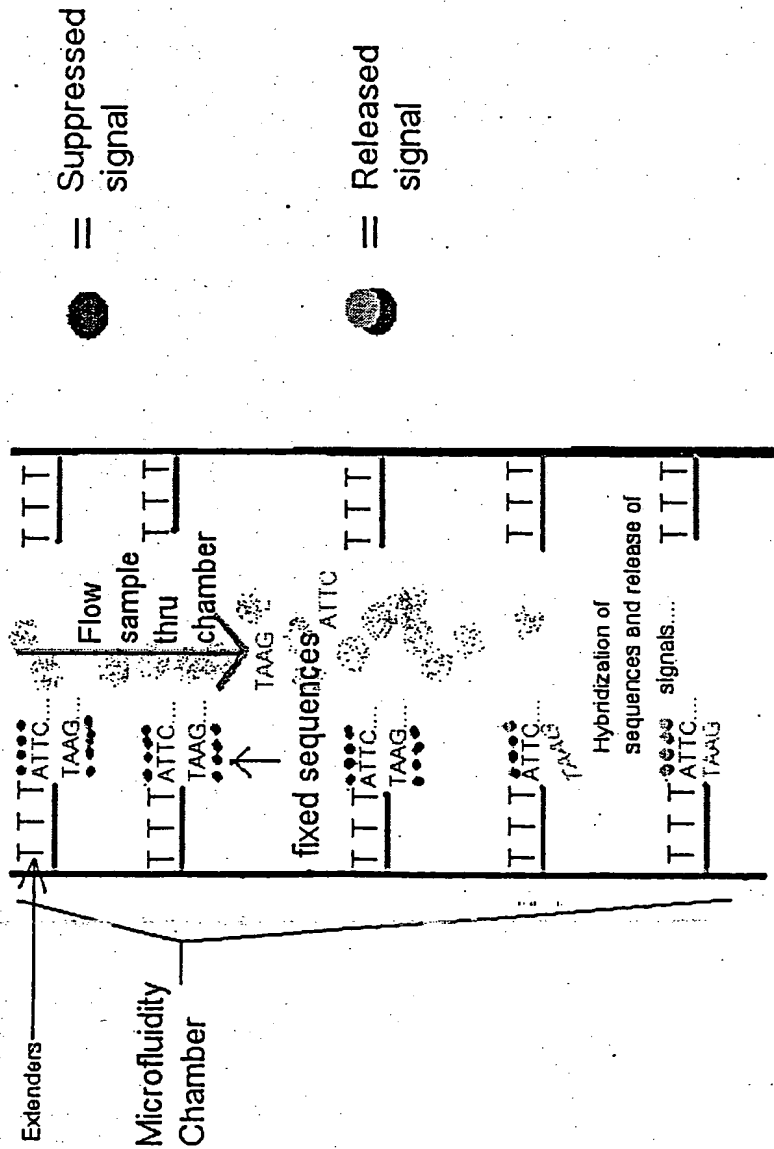


FIGURE 10

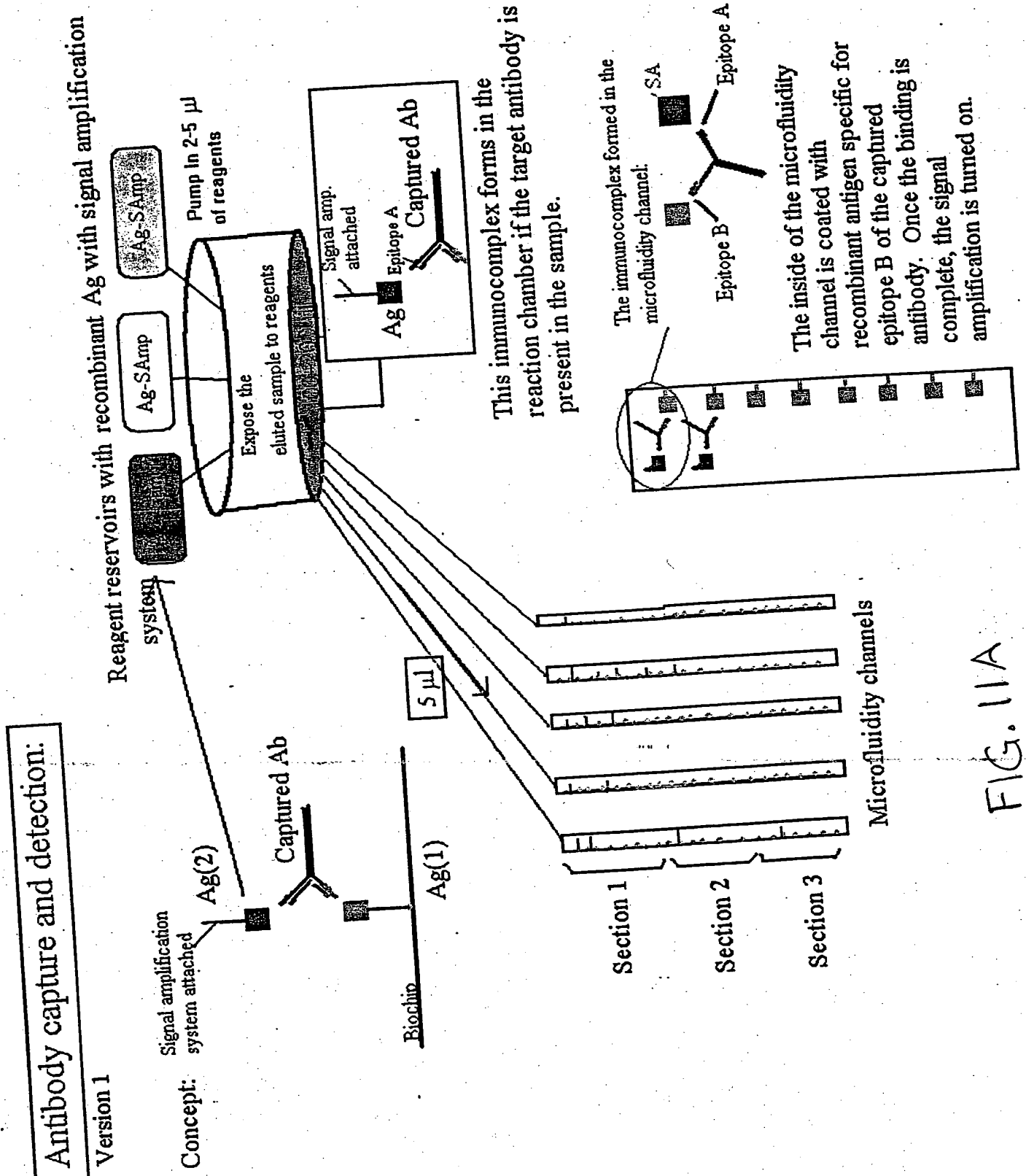


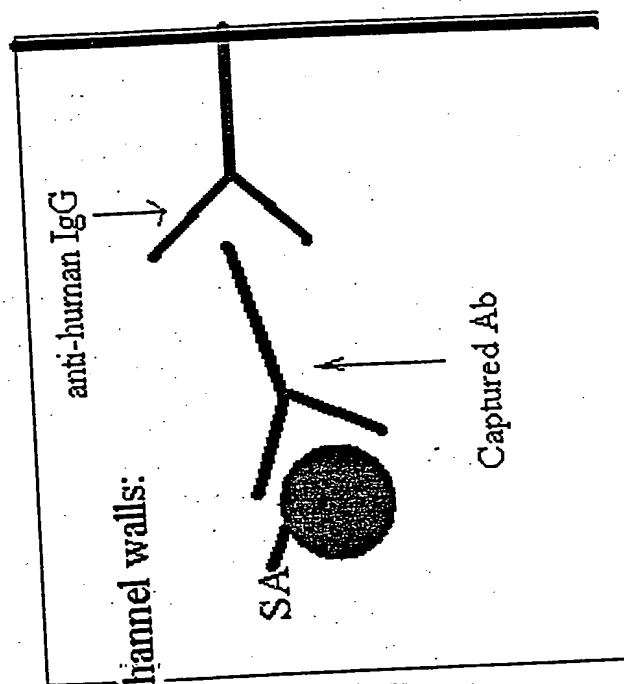
FIG. 11A

## Antibody detection

### Version 2

The eluted antibodies from the biochip will still be exposed to antigen reagents labeled with signal amplification systems to create an immunocomplex similar to that in Version 1. However.....

Instead of coating the microfluidity channels with recombinant antigen, we would coat them with an excess of anti-human IgG Fc specific for the captured antibody....the human IgG will recognize and bind to the Fc region of the captured antibody.



Immunocomplex formed on channel walls:

FIG. 11B

## Antibody detection Version 3

Instead of exposing the eluted disease antibodies to a labeled antigen reagent, we will expose them to an anti-human IgG Fc antibody labeled with a signal amplification system. The formed antibody:antibody immunocomplex will then be passed through a microfluidic channel coated with antigen specific to the disease antibodies. In the presence of disease antibodies, the below complex will form and detection is completed via signal amplification.

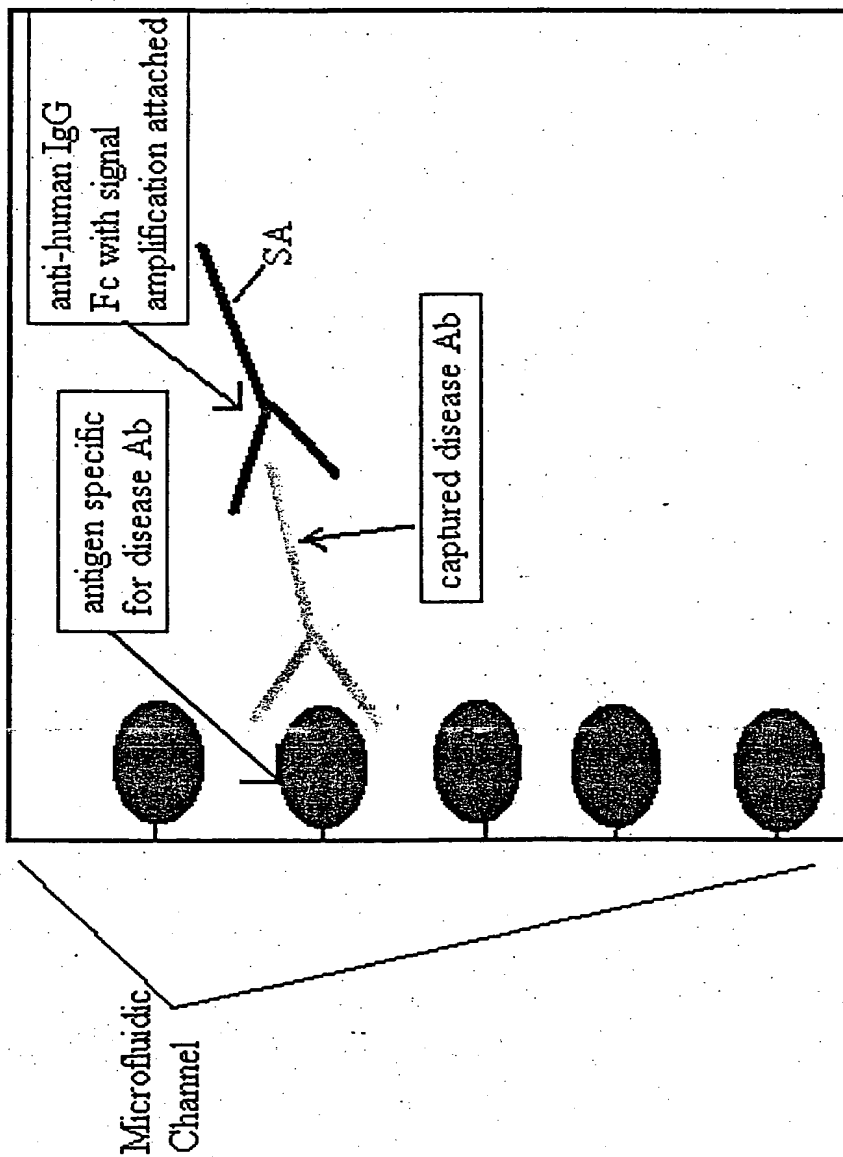
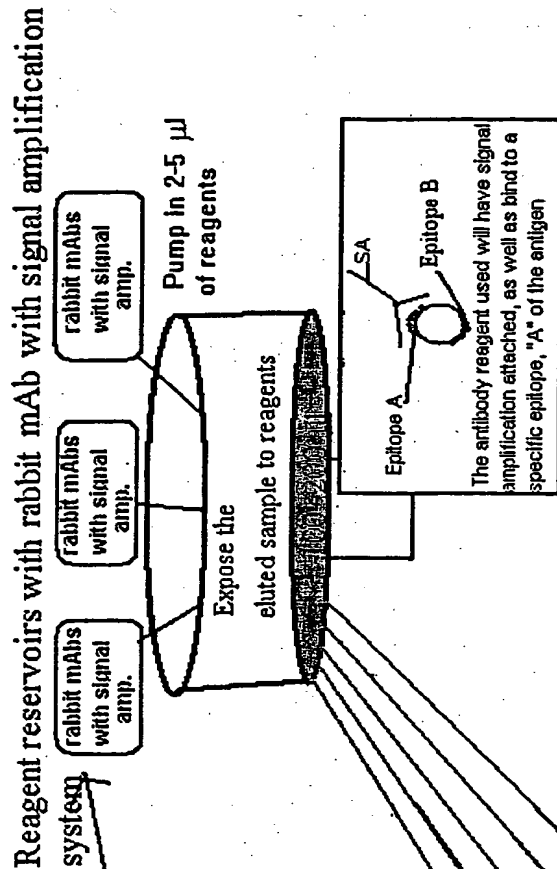
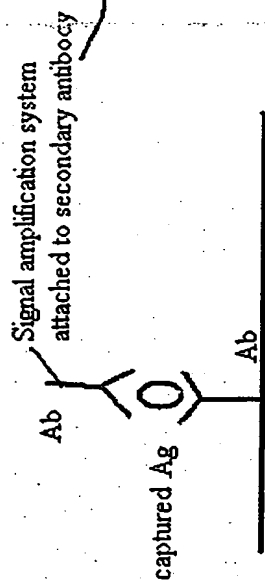


FIG. 11C

# Antigen Capture and Detection:

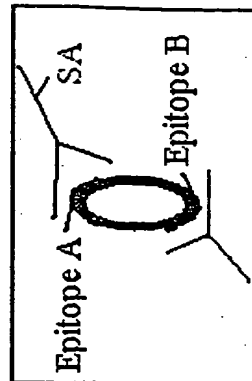
Concept:



This immunocomplex forms in the reaction chamber if the target antibody is present in the sample.

Mouse mAbs specific to epitope B of the captured antigen are coated on the walls of the microfluidity channels. No false positives

Immunocomplex formed in channel:



5  $\mu$ l

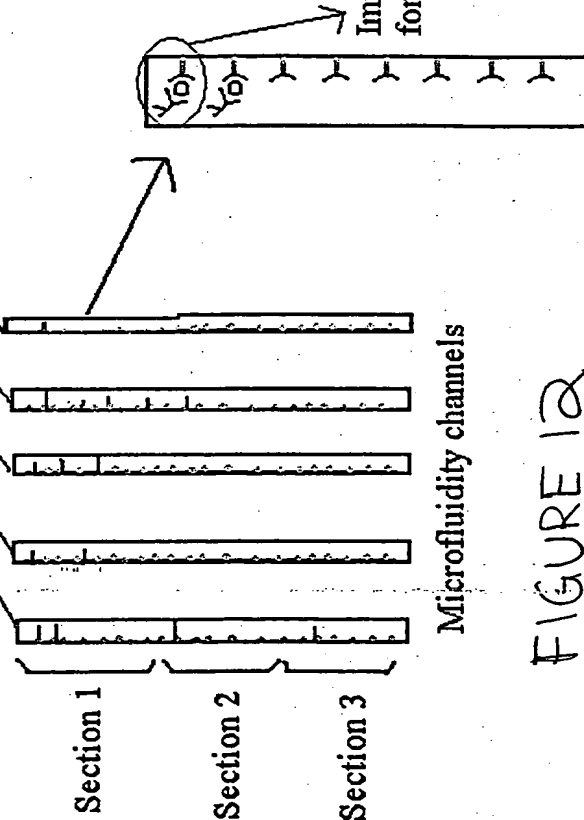
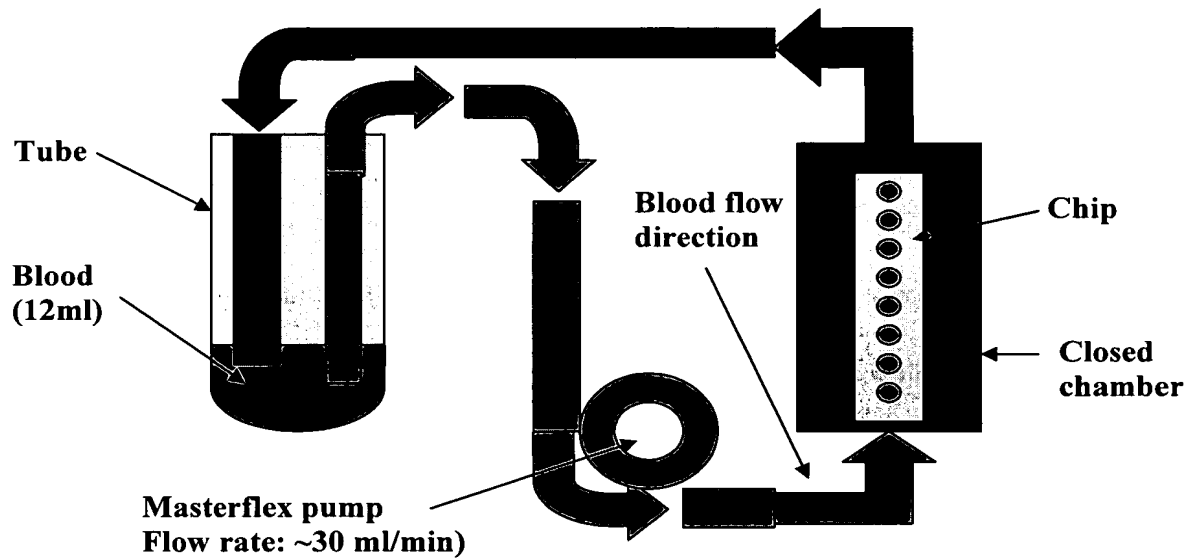


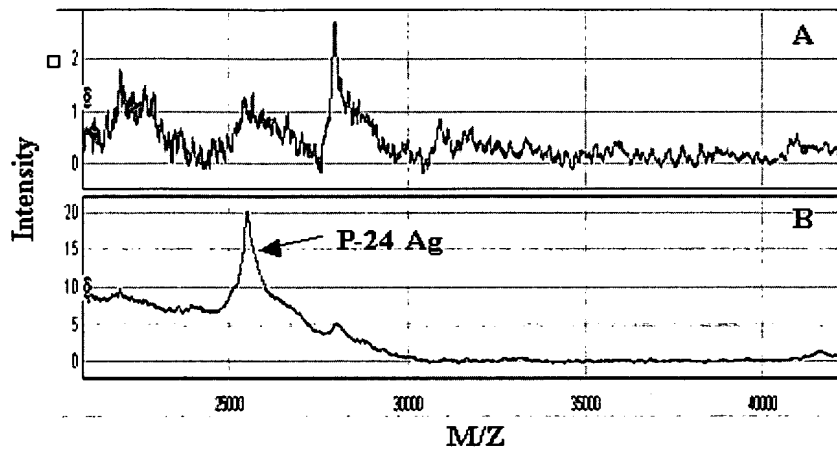
FIGURE 12

**Figure 13: Apparatus for capturing blood pathogen when the blood is flown through:**



**Figure 14: Capture antigen in blood.**

In this example, the chip was coated by bovine IgG (A) or monoclonal antibody against HIV P-24 antigen (B). P-24 antigen was added to blood, and was captured by the chip that was coated by monoclonal antibody (B) as shown in the graph, but not by bovine IgG (A).



**Figure 15: Capture antibody in blood.**

In this example, the chip was coated by HIV gp120 envelop protein (A) or HIV P-24 core protein (B). Monoclonal antibody (MonoAb) against P-24 was added to blood, and was captured by the chip that was coated by P-24 antigen (B) as shown in the graph,, but not by gp120 antigen (A).

